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Embryogenesis of Behavioral Nerve Nets

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The old problem of how all the information required to build a complete organism can be compacted and funneled through the microscopic dimensions of the zygote—and the problem involved in the reading out of all this genetic information, step by step, in development—is encountered in probably its ultimate and most challenging form in the developmental organization of the brain and nervous system, especially in the higher vertebrates. Here, in addition to the many problems of morphogenesis and cytodifferentiation common to other organs and organ systems, formidable enough in themselves, is the more complicated problem of the wiring of this whole system for behavior. Literally billions of individual nerve cell units must each acquire through their arboritic fiber extensions precise patterns of anatomical contact with dozens, and in some cases even hundreds, of other nerve cells—many of which lie at great distances from the parent cell body—thereby posing a truly enormous problem in the patterning of detailed interrelations between individual cells.

For a long time it was believed that the developing embryo avoided the whole problem simply by letting the growth process spin out a random, diffuse, unstructured, essentially equipotential transmission network; a blank slate as it were, leaving behavior, function, practice, experience, learning, and conditioning to mold and shape the fiber pathways into a functionally adaptive communication system. The old saying long prevailed: "Function precedes form in the development of the nervous system."

Many diverse lines of converging evidence reinforced this earlier view well on through the 1930s and into the early '40s. Today, by contrast, most of this supporting evidence has disappeared until now some researchers are taking an almost diametrically opposite stand—to emphasize that the great bulk of the behavioral circuitry of the nervous system, espe-

cially in infrahuman vertebrates, is built in. That is, the behavioral networks are organized in advance of their use, by growth and differentiation primarily, with relatively little dependence on function for their orderly patterning. The center of the problem has thus shifted from the province of psychology to that of developmental biology.

On analysis, the problem of the embryogenesis of behavioral nerve nets boils down in large part to questions concerned with the outgrowth and termination of the developing nerve fibers. Particularly critical for the adaptive patterning of functional networks is the matter of selectivity in the guidance and connection of the advancing fiber processes. The present discussion is centered on this selectivity problem to the necessary neglect of other material that would be both pertinent and important in a broader treatment of the subject. How do the thousands of fibers in a peripheral nerve trunk get connected, each to its correct type of end organ and in the proper location, and how do the even more numerous fibers of the central trunk lines manage to find their correct connection zones and the proper individual junctions within each terminal zone? A key concept in the more recent, more genetically oriented outlook on these matters, is that of the chemical differentiation or qualitative specificity of individual nerve cells and fibers and its influence on the formation of fiber pathways and connections. Much of what follows is concerned with the extent to which the cytochemical specificity of the individual nerve cells and fibers determines the kinds of structural linkages that neurons form.

Historical Background

The matter of selectivity in nerve growth has always been rather controversial and has a long history of pros and cons. This in turn has been closely intertwined with related physiological controversy centered around the functional significance of specificity in nerve connections. In the late 1930s when I was first attracted to these problems, the evidence seemed to be almost entirely against cytochemical specificity affecting the kinds of connections nerves form. Many studies of nerve growth and regeneration, both in vivo and in tissue culture, seemed to point to the conclusion that nerve fiber growth and termination are diffuse and indiscriminate.

It was reported, for example, that a skeletal muscle nerve would reinnervate and activate any other muscle as readily as its own muscle; that even sensory nerves would form functional connections with muscles, as would also their dorsal roots. Similarly, motor nerves would reconnect with sense organs, and peripheral nerves reflected into the spinal cord would form functional synapses in the spinal centers. Autonomic nerves would connect with somatic endings and vice versa; and a single motor axon, by successive branching, might innervate three, four, or more different nonsynergic muscles and still activate each independently in its own proper timing. A large number of examples could be cited, and the over-all picture was concluded to be overwhelmingly against any chemical selectivity in nerve growth (Weiss, 78–82).

Electrical selectivity also appeared to have been ruled out along with the chemical, in favor of the mechanical,

stereotaxic or contact guidance theory of nerve growth. This seemed adequate, along with various secondary factors like correct timing, differential growth rates, and ultrastructural alignments to explain satisfactorily the total developmental patterning of the brain pathways and connections (Weiss, 82). With appropriate reinterpretations, the mechanical view also appeared to account for earlier examples of supposed chemical and electrical selectivity cited by Cajal and others.

At the same time, and of critical concern for problems of the brain's function as well as its development, strong experimental support had been accumulated during the preceding 15 years for the conclusion that neuronal specificity has its effect, not on the kinds of morphological connections that neurons acquire, but rather on the kinds of signals that they transmit, and to which they selectively respond. Clinical reports as well as experimental observations covering vertebrates from primates to amphibians seemed to show that nerves not only regenerate and connect indiscriminately, but that the kinds of connections they form make little difference to the over-all function.

A series of studies on the surgical disarrangement of nerve-muscle connections in amphibians seemed to indicate that individual limb muscles continue to respond with the same timing, regardless of experimental rearrangements in the pattern of their nerve connections to the spinal limb centers. Each muscle in transplanted supernumerary limbs with deranged innervation would contract in perfect synchrony with its muscle homologue in the original limb (*homologous* or *myotypic* response). This phenomenon, which was found to prevail also in interchanged limbs, in transplanted individual muscles, and even after limb deafferentation and spinal transection (Weiss, 79–81), seemed to contradict the traditional idea of the reflex connection, and the all-or-none conduction principles of neurophysiology. It thus called for a new and different principle for describing selective communication in nerve networks.

The conclusion was being drawn that selective communication in the nervous system must be based, not on selectivity among synaptic connections, as the classical textbook doctrine would have it, but rather upon some kind of qualitative selectivity among the signals carried by different fiber types; that is, some kind of impulse specificity, or "Erregungspecifität," with selective resonance governing the pickup and firing from cell to cell. Schemes of this kind had been suggested earlier by Hering (25), among others, and had been more extensively formalized by Weiss in the resonance principle of nervous function (Weiss 76, 77, 79–81). In the resonance principle, the old telephone switchboard analogy was replaced by a radio broadcasting concept of communication, wherein selective reception and discharge of neurons was held to be independent of specific anatomical connections. The anatomical connections were inferred to be diffuse and nonselective. The issue of signal specificity vs. connection specificity had become critical both from the standpoint of brain function and from that of the developmental patterning of behavioral nerve nets. Its paramount importance was stressed above all others in the nerve growth literature of the 1930's.

In checking over the experimental data underlying the resonance principle and the doctrine of impulse specificity, it occurred to me that an alternative explanation had been overlooked which, if correct, would not only undermine the case for impulse specificity in favor of specific connections, but would at the same time reopen the whole question of chemical selectivity in nerve growth. This alternative explanation was first suggested to Professor Weiss in September 1938. The hypothesis was then outlined more formally during the next two years at the University of Chicago and has since survived a long series of experimental tests.

In brief, the proposal suggested that we completely reverse opinion on the question of chemical selectivity (Sperry 42–53, 55–57). Instead of following the prevailing view that formation of synaptic connections is diffuse and nonselective, it went to the opposite extreme to postulate that the formation and maintenance of synaptic connections take place with great selectivity, and that the whole process is strictly regulated by highly refined and specific chemical affinities between the individual neuronal elements present in any local brain center, neuropile region, or other synaptic zone. It indicated further that earlier attempts to forcibly switch nerve connections in the studies on homologous response in young amphibians had failed to accomplish the effects intended because the synaptic connections started rearranging themselves, in accordance with specific inductive effects in the new periphery and the new pattern of chemical affinities. The end result of the specific growth pressures is the restoration of essentially the same pattern of sensori-neuro-motor reflex linkages that existed at the start. Whether the selective regrowth occurs primarily in the centers or in the periphery is something of an open question at present. Either way the relevant conclusions are the same.

This chemoaffinity interpretation not only offered an explanation of the perplexing 18-year-old problem of homologous response and related phenomena, obviating the concept of impulse specificity, but it also provided the basis for an entire new and attractive hypothesis for the normal developmental patterning of behavioral nerve nets. By incorporating some of the current concepts of experimental embryology such as morphogenetic fields, gradients, and organizer and induction effects, and applying these to the functional wiring of the sensori-neuro-motor system in the chemoaffinity context, it was possible to set up a fairly comprehensive approach to the ontogenetic patterning of behavioral nerve nets. This proved to be much more satisfactory, detailed, and explanatory than any of the ideas previously available, such as neurobiotaxis, disuse atrophy, contact guidance, bioelectric fields, functional trial and error, conditioning, and so on.

Subsequent experimental tests of the hypothesis have been almost uniformly confirmatory. The first promising result came from interchanging motor and sensory nerve relations in 3- to 5-week-old rats (Sperry, 41, 42, 43; Weiss and Sperry, 87). Contrary to expectations and to the voluminous earlier literature, pronounced disfunction effects were obtained. They were exactly the kind that would be expected if selective communication in the nervous system were based on specific nerve connections patterned by growth and

subject to little or no rearrangement by re-education. The whole idea of the preceding half-century—that nerve connections are functionally interchangeable—was, I finally concluded (Sperry, 49), simply a myth. The switching of nerve connections to sensory and motor end organs, in man, or any other mammal, produces corresponding distortions of function. This does not apply to the lower vertebrates, particularly to larval salamanders, largely because the embryonic forces for organizing the sensori-neuro-motor hookups remain operative, and are very effective in restoring, in one way or another, the original connection patterns.

More direct tests for selectivity in synaptic formation were undertaken during the next 10 years in lower vertebrates where many central fiber systems were found to be capable of functional regeneration (Sperry, 43–57). Surgical section, scrambling, and transplantation of central fiber tracts and the transplantation of nerve and end organ relations during development led, in all cases, to functional results that supported directly the deduction that the terminal connections of the growing nerve fibers are laid down in a highly selective fashion as postulated; and that these connections are governed by the intrinsic specificity of the advancing fiber tip plus that of the various cellular elements it encounters in its outgrowth.

New Dimensions in Neurospecificity

The initial experiments carried out on the optic nerve disclosed the existence of new orders of refinement in neuronal specificity, so refined as to approach the level of the individual nerve cell and its axon fiber. Even in the resonance principle of Weiss it had seemed unreasonable to imagine a neurospecificity so refined as to provide a different signal quality or specific neuron type for each different direction in visual space—or similarly, for each different point in the cutaneous field. It had been assumed, accordingly, that these directional attributes were probably installed on some other basis (Weiss, 77). Additional dimensions of specification in the population of the optic fibers were later to be inferred with regard to the perception of colors and luminosity and with the different "on-off" fiber unit types described by Hartline (23) and others (Maturana et al., 32) that must presumably be involved in optokinetic responses to moving stripes, in striking at small targets, and in some of the pattern perception tests (Sperry, 55; Arora and Sperry, 3, 5). By the late 1940s the evidence (Sperry, 52, 53; Stone, 67; Sperry and Miner, 66) was sufficient to suggest, as a basic principle, that neuronal specificity and interneuronal affinities operate generally throughout the nervous system with an order of refinement that parallels closely the inherent functional differentiation.

Changing Views

Meantime, with the advent of the chemoaffinity interpretation, the campaign for impulse specificity was promptly abandoned. By 1947 even the strongest opponents of chemical selectivity, swayed increasingly by the above and other evidence (see Holtfreter, 26), had come well around toward a reversal of their earlier position of the 1930s (Weiss, 83). This turnabout of opinion did not come easily

after the heavy investment of the preceding two decades against both chemical selectivity on the one hand and fiber connection specificity on the other. It was accomplished in the literature more by verbal subtleties than by any frank acknowledgment of a change or correction of earlier views. As a consequence, the literature since 1939 has become something of a tangled web of ambiguity and forced terminology that is understandably perplexing to the novice not acquainted with the underlying history.

For example, "chemotaxis" and "chemotropism" were first attacked on an antiselectivity basis, but when this no longer seemed wise, the terms were carefully defined (Weiss and Taylor, 88) so as to include the concept of attraction from a distance, and from then on the attack was shifted to the implied distance effects. Instead of chemotaxis and chemotropism, terms like "selective contact guidance," "preferential contact affinities," and the like are used. After the renunciation of Cajal's concept of "selective attraction" it is allowed that there may be selective "proximity" effects in addition to those of direct "contact" (Weiss, 83). "Selective fasciculation" was used initially to describe the tendency of any fiber to follow along those particular preceding fibers that had happened blindly to succeed in achieving a terminal connection, whereas now it is coming to be used more and more to mean the selective segregation in growth of similar fiber types as conceived earlier by Cajal (37, 38). The old terms "stereotropism" and "stereotaxis" mean "guidance through contact with solid surfaces" like contact guidance. But if the aim is to impute distance effects in one's definition of tropisms and taxes, it is better not to remind the reader of "stereotaxis" or "thigmotaxis."

Specificity in fiber connections was denied for many years as a basis for selective communication in nerve circuits. When the ground started to become shaky on this point, the attack again was not dropped or corrected, but was shifted to decry the notion of absolute invariance in the development of anatomical relations, something that never was much questioned and is largely irrelevant to the issue. The term "resonance" originally used to suggest that muscles respond selectively to specific impulse frequencies even where asynchronous muscles have been innervated by separate branches of the same axon (Weiss, 76, 77), has survived to the present, but only by undergoing repeated metamorphoses of meaning until the original sense is long lost.

Meantime the actual experimental observations showing lack of selectivity in the growth of nerve connections, no longer in accord with the changed outlook, have had to be retracted, contradicted, or strongly qualified (Weiss, 84). The remainder of the findings of the 1930s along this line, including even those indicating a nonselective reinnervation of skeletal muscle by skeletal motor axons, are today open to question and re-examination (see p. 176).

During this same period, when the resonance concept and impulse specificity were being abandoned in developmental biology, the reverse process was going on in electrophysiology, where the general uniformity of nerve action potentials made the postulation of hundreds of thousands of chemically specific fiber types seem a bit difficult to accept. Lettvin and others have ar-

gued for years, and correctly so, that the orderliness of the functional recovery in optic nerve experiments and similar ones, does not necessarily prove a selective orderliness in fiber reconnection (Lettvin et al., 30). The optic fibers, it was said, might carry different types of signals or pulse patterns of some sort, which could then be decoded at the central stations even though the central connections were quite randomized. This too was largely abandoned after Gaze reported in 1959 (11–13) that the projected map of the retina on the brain after optic nerve regeneration was the same as before the nerve was cut. This was confirmed within the year by Maturana, Lettvin and co-workers in their own microelectrode studies (Maturana et al., 32). They reported further that they were able to record separately the several types of fiber terminals pictured in the tectum by Cajal (38) and that each type regenerated selectively to its normal depth and locus in the tectum.

With regard to mapping by electrical methods, however, it has been pointed out (Gaze, 14; Attardi and Sperry, 8) that it is difficult to be certain whether one is recording the topography of the regenerated fibers, or just the more physiologically effective portions thereof; or perhaps not the regenerated fiber system at all but rather the postsynaptic potentials and discharges in the tectal neurons. Further, the course which the regenerated fibers traverse within the amphibian tectum en route to their terminals cannot be traced by the present electrical techniques. Hence the electrical evidence, like the

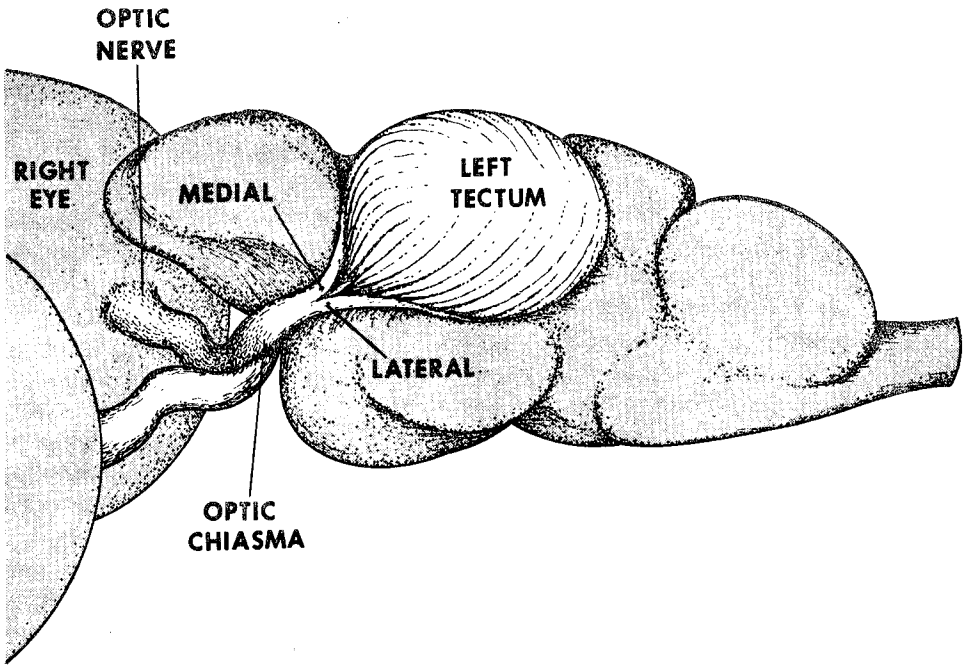


Fig. 6.1 Schematic drawing of goldfish optic system showing division of main optic tract into medial and lateral bundles and their relations with midbrain tectum. (After Attardi and Sperry, *Exptl. Neurol.* 4:262–275, 1961.)

behavioral, is not in itself enough to rule on the question of impulse specificity and signal decoding vs. selective regrowth. What was needed to settle such questions, as pointed out by Gaze (14), was direct histological evidence.

Bodian silver stain studies of optic nerve regeneration have been carried out in fishes in which the optic system in general is more refined and more highly elaborated than in the amphibians (see Fig. 6.1). These results (Attardi and Sperry, 7, 8) provide finally a direct and convincing histological demonstration of strong chemoaffinity effects in regrowth of the optic fibers, not only with respect to the terminal connections that they establish in the tectum, but also with respect to the central pathways which the fibers take to reach their terminal sites (Fig. 6.2). The selective growth patterns occur, in this case, under conditions where the possibility of alternative mechanical interpretations seems highly remote.

Ever since the early studies of Harrison (22), it has been commonly agreed that nerve fiber growth is universally subject to contact guidance. Nerve fibers have to grow in contact with surfaces and interfaces of some sort; they cannot grow directly across empty liquid or gas spaces. The issue has remained as to whether, in addition, the direction of growth is influ-

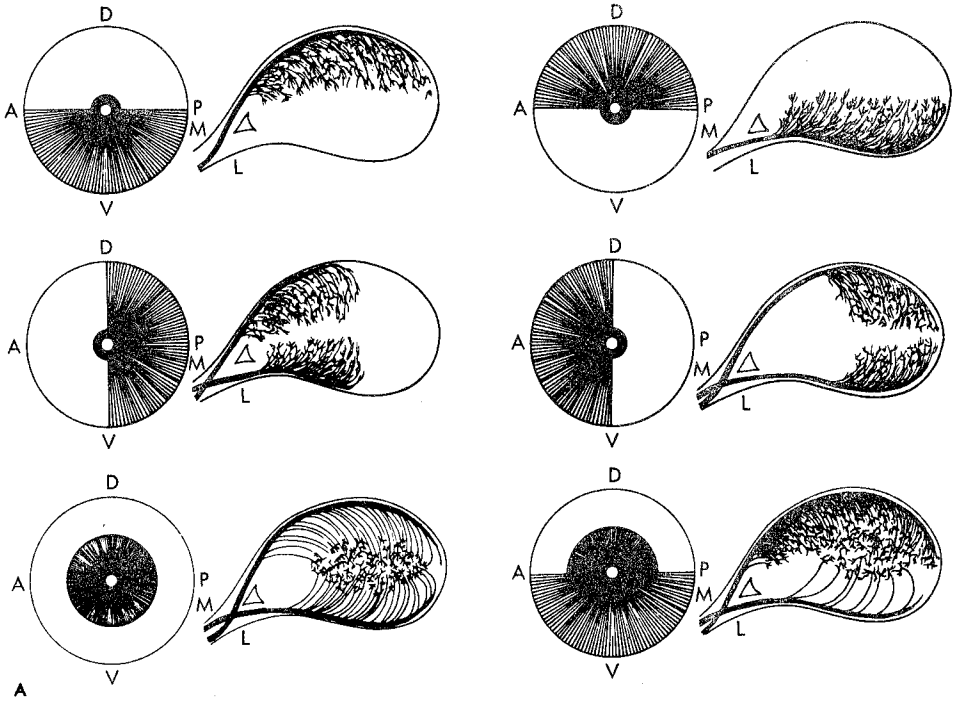
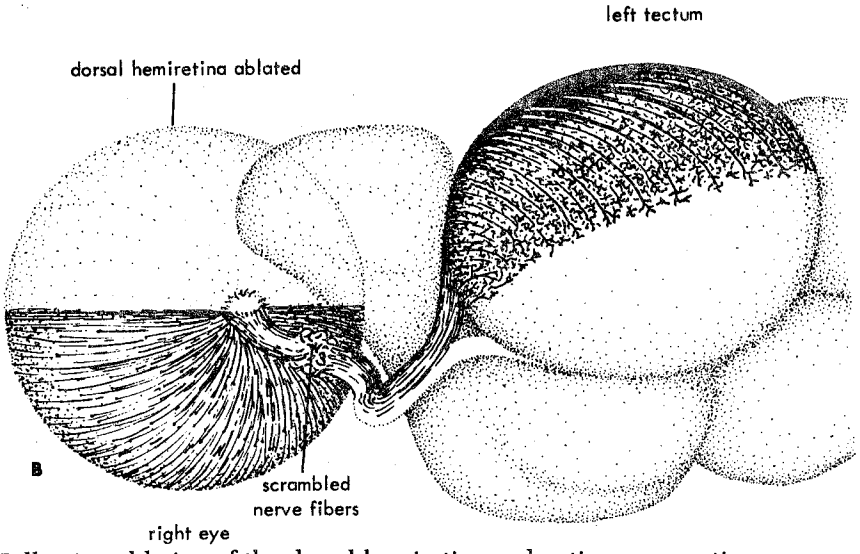
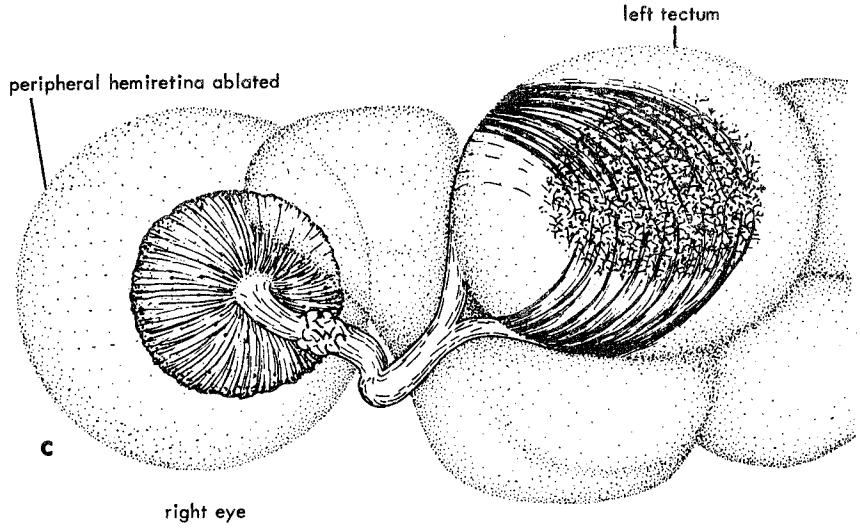


Fig. 6.2 Results of experiments in which the dorsal, ventral, anterior, posterior, or outer peripheral hemiretina is excised, after scrambling of fibers in the optic trunk. (After Attardi and Sperry, *Physiologist* 3:12, 1960; *Exptl. Neurol.* 4:262–275, 1961.) *A.* Diagrammatic reconstruction of regeneration patterns formed in the optic tracts and tectum by fibers originating from different retinal halves.



B. Following ablation of the dorsal hemiretina and optic nerve section, regenerating fibers from the ventral retina form synaptic connections only in the dorsal tectum. (After Sperry, *Proc. Natl. Acad. Sci. U.S.* 50:703–710, 1963.)



C. Fibers from the center of the retina grow through the denervated peripheral zone of the tectum, but establish synaptic connections when they reach the central tectum. (After Sperry, *Proc. Natl. Acad. Sci. U.S.* 50:703–710, 1963.)

enced also by selective chemical factors (chemotropism) or by electrical factors (galvanotropism), or whether the purely mechanical contact factors (stereotropism) are sufficient, as contended in the mechanical theory. Because the mechanical elements are universally present along with possible chemical factors, it has always been extremely difficult in any given

situation to prove the existence of chemical selectivity, especially with the weight of opinion so long against chemical interpretations. In our current experiments, the mechanical conditions are seemingly constant, but fibers from different sectors of the retina show very different growth patterns in the same mechanical matrix.

The availability now of a seemingly unequivocal demonstration of the presence of strong chemical selectivity, in respect to both the course and the termination of the optic nerve fibers, has opened the gates for similar interpretation of many other borderline observations where chemotropic explanations of nerve growth had long appeared possible but had been withheld or were considered suspect because of the dominant long-term bias against anything suggestive of chemotaxis, chemotropism, or neurotropism. In the numerous papers of Hamburger, Piatt, Speidel, Weiss and other students of nerve growth, one can find repeated observations where selective chemical affinities would appear, in retrospect and from the vantage of present evidence, to offer a reasonable and perhaps now the most probable explanation (Hamburger, 20). The problems involved in the directional guidance of a migrating nerve cell would appear to be similar in many respects to those that arise in the advance of the fiber growth tip. The orderly cell migrations described in the beautiful studies of Levi-Montalcini (31) come immediately to mind in this regard.

The Current Thesis

We can turn now to a brief statement of the general working picture as it stands today without dwelling further on the supporting published evidence that can be found in detail in the preceding and later references (see Miner, 33; Sperry, 58–61, 63; Szekely, 71, 72).

It now appears that the complicated nerve fiber circuits of the brain grow, assemble, and organize themselves through the use of intricate chemical codes under genetic control. Early in development the nerve cells, numbering in the billions, acquire, and retain thereafter, individual identification tags, chemical in nature, by which they can be recognized and distinguished one from another.

As the maturing neurons and their long pulse-carrying fibers begin to form functional interconnections to weave the complex communication networks of behavior, the growing fibers become extremely selective about the chemical identity of other cells and fibers with which they will associate. Lasting functional hookups are established only with cells to which the growing fibers find themselves selectively matched by inherent chemical affinities.

The outgrowing fibers are guided by a kind of probing chemical touch system that leads them along exact pathways in an enormously intricate guidance program that involves millions and perhaps billions of different chemically distinct brain cells. By selective chemical preferences the respective nerve fibers are guided correctly to their separate channels at each of the numerous forks or decision points which they encounter as they travel through what is essentially a multiple Y-maze of possible channels (Fig. 6.3).

Each fiber in the brain pathways has its own preference for particular prescribed trails by which it locates and connects with certain other neurons

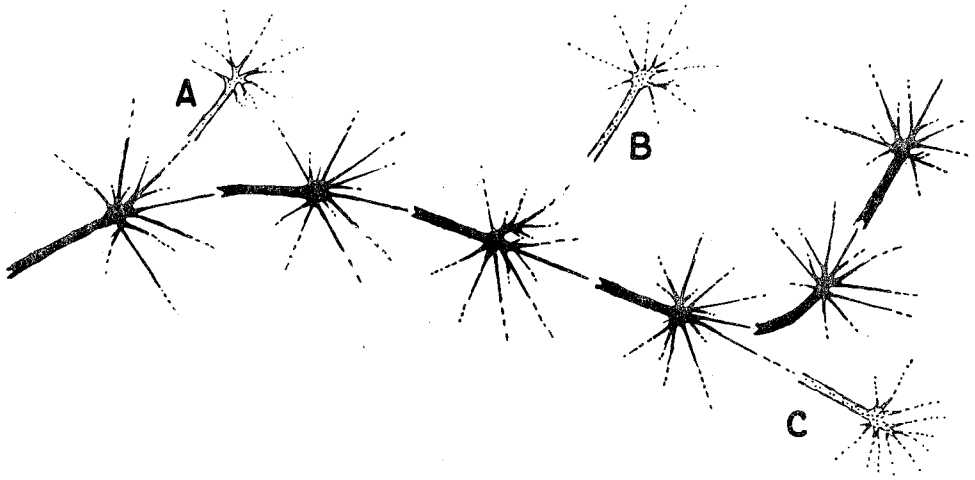


Fig. 6.3 Schematic representation of sequential steps in chemotactic guidance of a growing nerve fiber. Multiple filopodia constantly reach out in front of the advancing fiber tip, testing the environment in all directions. The critical factors determining which filopodia will form the strongest attachments, and thereby direct the route of growth, are apparently chemical. Numerous alternative paths, like those represented at A, B, and C, are open, mechanically feasible, and may even be established temporarily, but fail to survive because of differential selective adhesion.

that have the appropriate cell flavor. The potential pathways and terminal connection zones have their own individual chemical flavors by which each is recognized and distinguished from all others in the same half of the brain and cord. Indications are that right and left halves are chemical mirror maps of one another.

The present scheme provides the basis for a general explanation of how instincts and other inherited components of behavior, of even the most detailed sort, can be ingrown. Limitations in the machinery of growth are largely removed in this view in which the developmental mechanisms are believed to be capable of handling the most highly refined and precise adjustments in the neural networks. In regard to the inheritance of a given behavior pattern, it is no longer so much a question of whether the machinery of growth is capable of installing it, as to whether the survival rate may be better if the behavior is kept flexible by having it learned in each generation and thus adaptable to external conditions and adjustable to changes. The fact that in any individual the particular constellation of chemical affinities that emerges within the tremendous population of developing neurons happens to yield connection networks that are functionally adaptive goes back to the selection pressures that affect survival throughout evolution. How the process of neurochemical evolution is controlled in ontogeny remains one of the many challenging problems of developmental biology.

It is apparent that our current view, with its emphasis on chemical selectivity, comes closer to the older ideas of Cajal and his contemporaries than to the antichemical, antiselectivity views

prevalent in 1940. The course of the selectivity theory, however, has not been so much a return full circle to the earlier position, perhaps, as it has been a hairpin curve or switchback in the upward advance of the field. The stress on physicochemical forces in nerve growth led by Weiss (84) over a period of three decades has been most catalytic and instructive. This whole important aspect of the picture is omitted in the present discussion focused on the selectivity issue. Earlier proponents of chemotaxis and neurotropism had applied these concepts in neurospecificity to the peripheral nervous system principally, mainly to nerve-end-organ relations. Even Cajal had been willing to leave for functional assistance the detailed patterning of the central connections for coordination, perception, sensory local sign, and the like. All of these, in the present interpretation, are believed to fall within the province of growth and differentiation. Although the present scheme allows ample room for learning and memory, it represents a strong swing during the past 25 years toward an increasing recognition of the importance of inheritance in behavior. How far the pendulum may continue to move in this direction remains an open guess.

In no other part of the central nervous system do the cell-to-cell associations have to be installed with greater selectivity and precision than in the sensory pathways and brain centers for vision. Application of our current theory to the human visual system requires that the one million or so fibers that connect eye to brain be individually tagged, each fiber distinguishable according to the latitude and longitude of its point of origin in the retina. We infer that gradients of embryonic differentiation, with their axes essentially perpendicular to each other, spread across and through the developing tissues, to impress the required chemical mapping on the embryonic retina and optic centers of the brain. Three of these morphogenetic gradients, superimposed, would be enough for the topographic mapping involved and would give corresponding values for identical points in the retinal fields of each eye and between the retinae and the series of brain centers on each of which the retina is mapped by orderly fiber projections. Nasal-temporal differences at the retina and corresponding right-left cytochemical factors in the centers preserve distinct image paths from each eye with right and left maps in register at each central station, shown in Figure 6.4 (Sperry, 63). The same kinds of processes visualized here with reference to the optic system must go on in all parts of the developing nervous system.

Remaining Problems

The Underlying Chemistry

Rather than review the already published evidence on which the foregoing generalizations are based, it seems more profitable to comment on some of the unsolved problems and the current issues and questions still under investigation. Probably the most obvious question concerns the nature of the biochemistry of the specificity factors that underlie the demonstrated selectivity in nerve growth and other neuroaffinity phenomena. This remains, of course, an open field, practically untouched as yet, with relatively little in the way of evidence to curb creative speculation. The biochemistry in-

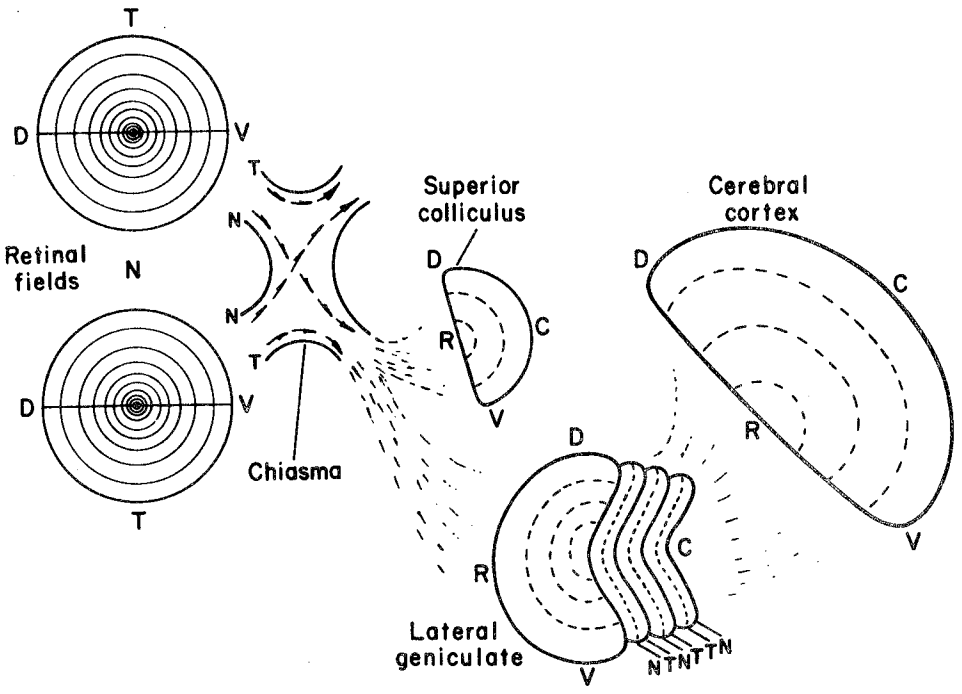


Fig. 6.4 Schematic diagram indicating possible application of chemoaffinity interpretation to genesis of mammalian visual system (see text). Axial labeling of gradients for brain centers is highly tentative because the effective embryonic gradients underlying their topographical differentiation remain uncertain. D–V, dorsoventral gradient; N–T, nasotemporal; R–C, rostrocaudal gradient. (After Sperry, *Proc. Natl. Acad. Sci. U.S.* 50:703–710, 1963.)

volved here is presumably that of cell differentiation carried to extreme refinement within the nerve cell population. Note that three basic axial gradients of differentiation—rostrocaudal, dorsoventral, and radial or mediolateral—would be sufficient to impress a unique chemistry on every single cell of the CNS, and of the entire body for that matter, depending on the steepness of the gradients. As in the differentiation of the organism as a whole, we may presume that many local fields of differentiation and subfields and subgradients are superimposed upon the three primary axial fields. These will be combined presumably with mosaic, frequency distribution and other forms of differentiation involving suppressive emanation, lateral inhibition, and the like. Differentiation within the CNS may be seen to reflect in more ways than one the differentiation of the total organism in miniature. The chemical problems in principle are presumably of similar nature.

The body and most of its parts are represented in the nerve centers in miniature and in functional perspective, several times over in some instances. For example, the entire body surface in full detail is represented at the spinal and hindbrain levels, again in the thalamus and again in the cerebral cortex. The face, head, and neck, at least, are doubly represented with duplication of right and left sides at thalamic and cortical levels (Fig. 6.5).

Cytochemical differentiation in terms of gradients and fields is basic all through the central nervous system. Cells close together within a given nucleus or cortical area are similar and those farther apart are increasingly dif-

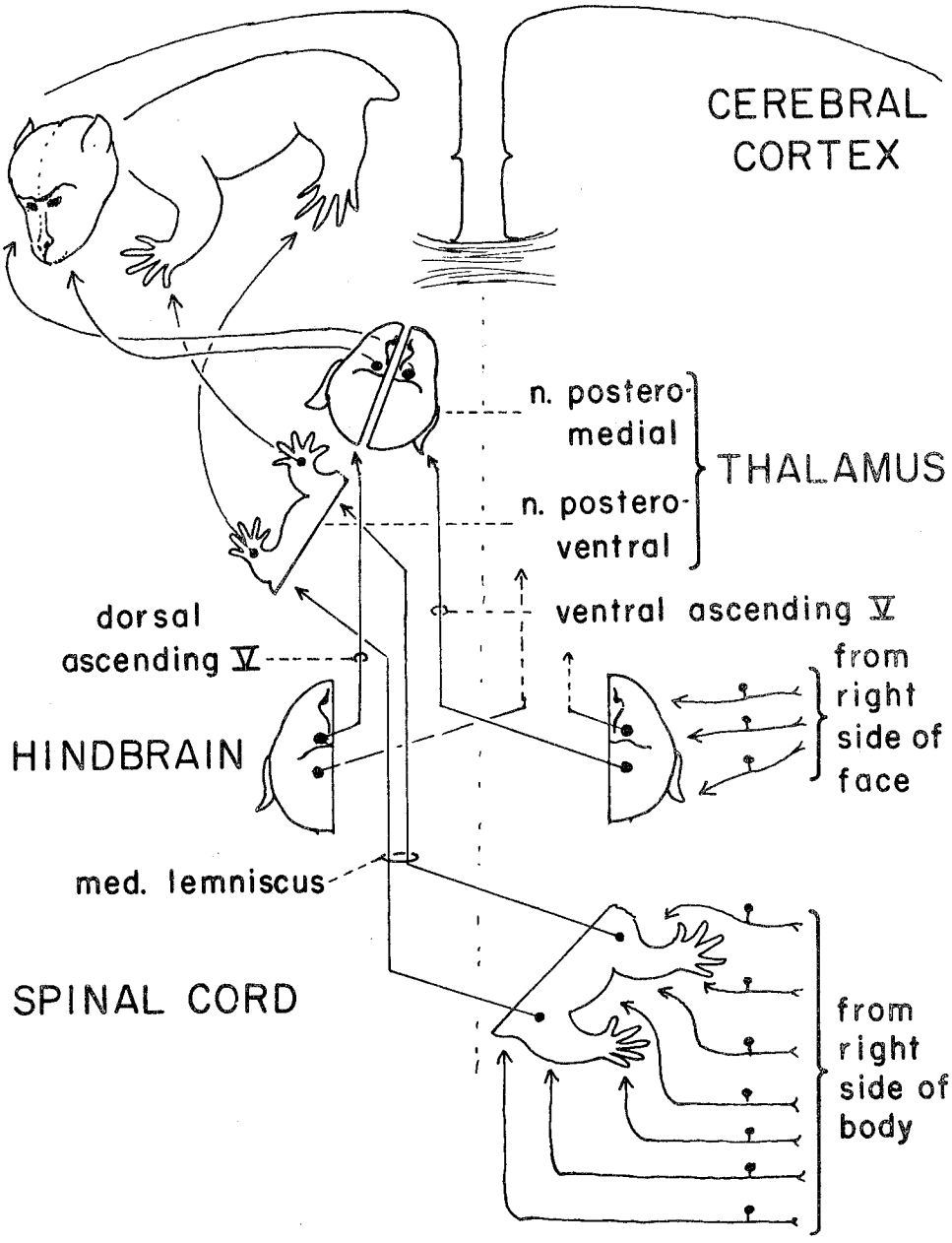


Fig. 6.5 Diagrammatic scheme to show neural representation of body surface in the spinal and hindbrain nuclei, thalamus, and cortex. In neurogenesis, similar differentiation takes place in many other pathways and centers throughout the entire nervous system.

ferent as the separation increases. The tendency for fiber projections from one central field to another to interconnect opposite poles of the two gradients appears so frequently in central nervous organization as to suggest that this affinity between opposites may be a rather direct reflection of the nature of the underlying chemical affinities, a complementarity principle perhaps (Jehle, 27). The dorsal retina, for example, connects to ventral tectum, the lateral somatic thalamus connects to medial cortex, the medial nucleus gracilis projects to lateral thalamus, and so on.

The nature of the gradients and their interrelations via fiber projection systems suggests a chemical basis in which some large molecule or molecular complex or unit has a long range of properties running from one extreme to another with many graded intermediate steps, each one of which is precisely controlled and precisely replicated from within a given cell. The chemical factor is then extended without loss of specificity into all the distant fiber tips of the given neuron (Sperry, 58). As a simplified hypothetical example, imagine a dipole compound molecule made up of units A and P (for anterior and posterior). At the rostral pole there are 99 A units to every one of P and the reverse at the caudal pole with a 50:50 ratio in the center. Once the over-all gradient is established, each unit ratio would become stamped into the differentiation machinery of the neuron and the specific factor transported throughout its arboreal fiber system. As indicated above, most neurons would also be synthesizing additional specifying molecular complexes for the dorsoventral alignment and another for the mediolateral alignment, plus further nongradient factors like those for color in the retina and for pain and temperature in the cutaneous system.

Though it is entirely possible that the specifying chemical factors become implanted throughout all parts of the nerve cell membrane, it is the soma of the neuron and the axon tips that are especially critical in determining communicative relationships. There are reasons for supposing that the membrane at the fiber tip is specialized not only in the active filopodial flare at the growth tip, but also in the comparatively quiescent synaptic junctions at end organs in the periphery and at end bulbs in the centers. There must be interchange and interaction of cell specificity effects through the fiber tip and soma, including the dendrite system. These effects seem not to be present and frequently are contraindicated along the length of the axon. Insulation of the axon by the myelin sheath is generally thought of in terms of impulse-conduction properties or nutritional processes, or both. The myelin sheath cell complex may also serve an insulating or related effect in reference to the establishment, maintenance, and preservation of chemical specificity.

The specialized cell-to-cell relations that exist in the nervous system, particularly in respect to the long fiber connections between distant cells, offer special analytic advantages for approaching the biochemistry of the morphogenetic field, of induction effects, and of other general features of growth dynamics. The chemical factors involved in the retinal gradients, for example, must be of such nature that they can be extended the full length of the growing axon into the fine probing

spiculelike filaments at the advancing fiber tip. They must be present and operating in the active microfilament surface. This would seem to suggest that the chemoaffinity is not dependent on specific patterning of molecular latticework in the surface membrane, for such a pattern would seemingly be difficult to maintain in a rapidly elongating spicule or film. Specificity within individual molecular units, regardless of their interunit patterns, would seem at first sight a more promising hypothesis.

The morphogenetic fields of the nervous system involve populations of contiguous cells as in organogenesis elsewhere. In addition, however, the specific cytochemical properties of the individual units of the field can then be extended and scattered in various ways over long distances and into widespread fiber arborizations. The unit properties are retained in these fiber systems which may themselves become randomly scattered, or rotated, or inverted on one or another axis, or duplicated by axon bifurcation, while the parent cell bodies have to maintain their proper positions in the field. Induction effects also can be mediated through the distant fiber contacts. Whereas, in organogenesis generally, differentiation induced by neighboring elements tends to be local and confined to neighboring cell masses, induction via fiber tip contacts permits distant inductive effects such as that of the integument on the spinal ganglia and dorsal columns (Sperry, 56; Miner and Sperry, 34; Miner, 33).

Innervation of Skeletal Muscle

It is a general impression in the literature that the growth and regeneration of nerves into skeletal muscles is fortuitous and nonselective (Weiss, 80; Weiss and Hoag, 86; Bernstein and Guth, 9; Guth, 16). Earlier demonstrations that even sensory fibers will innervate skeletal muscle has been retracted (Weiss and Edds, 85). Whether this nullifies also the conclusions drawn from observations on the mononeuronal reflex arc (Weiss, 78) is not clear. Our own studies emphasize that there is in actuality a great deal of selectivity in the innervation and reinnervation of muscle. We have found a wide variety of selectivity effects in different vertebrates and in different neuromuscular systems (Sperry, 42, 51, 54; Sperry and Deupree, 65; Arora and Sperry, 2, 6). These range from a muscle's complete acceptance of foreign fibers with full functional recovery, to complete rejection of foreign innervation, resulting in atrophy and degeneration of the muscle confronted with foreign nerves (see also Eccles et al., 11). Presumably there are many forms and degrees of neuromuscular selectivity yet to be clarified.

In recent studies (Arora and Sperry, 6) we found that the completely severed oculomotor nerve regenerates selectively in the cichlid fish *Astronotus ocellatus* to restore normal movement of the eyeball. Forcing individual branches of the nerve into the wrong eye muscle by surgical means leads to some reinnervation and weak contraction timed to suit the transplanted nerve but not its new muscle; that is, myotypic response is lacking. We concluded that the chemical specificities involved favor the original reconnections over the abnormal connections. Similar results have just been demonstrated in our laboratory by Mark in studies on reinnervation of the pectoral fin of the same fish. Mark suggests

that selective functional recovery in these lower vertebrates is associated with the multiterminal type of innervation. This permits competition of numerous fiber terminals, whereas in the other type of muscle the first fiber to enter, as a rule, captures the muscle and prohibits further competition. In other studies on reinnervation of salamander limbs after nerve cross-union, Mark found so much diffuse sprouting of the manipulated nerves that he decided it was almost impossible to prevent the regenerating fibers from getting back to their original muscles. These recent demonstrations of selectivity in the reinnervation of muscle have caused us to wonder about the claims regarding nonselective reinnervation in the "supernumerary limb" experiments and other early studies. Certainly our own earlier examples of myotypic specification of nerve by muscle (Sperry and Deupree, 65; Arora and Sperry, 2) need now to be rechecked. The critical observation on which the resonance theory of nerve function was founded and supported for over 10 years (Weiss, 76, 77, 79), namely, that a single motor axon can branch into several different asynergic muscles and activate them individually each in its correct timing, also needs to be re-examined in the light of our present knowledge.

In studies of salamander forelimbs transplanted into the opercular region, Hibbard, in our laboratory, has confirmed Detwiler (10a) to the effect that certain muscles of these limbs respond in synchrony with swallowing. The results seem to be an exception to the general rule that the musculature of transplanted limbs must be connected to limb centers in order to function, and that the muscles respond only at the time proper for the given muscle (Weiss, 84). One may tentatively infer that there is some fortuitous chemoaffinity involved between the particular plantar flexor muscles of the forelimb and certain of the nerves of deglutition. In an impressive series of transplantation studies in chicks and salamanders, Szekely and Szentagothai (69–74) obtained a number of results that they found difficult to account for by current theory. In a recent interpretation (Szekely and Szentagothai, 74) they consider a tentative return toward some kind of impulse-specificity scheme reminiscent of the resonance principle of Weiss in which the selective response effects are suggested to be independent of specific fiber connections. Their findings provide new information about the dynamics of neuron specification and the adjustment of nerve-end-organ relations, but it is not as yet clear that they are incompatible with the chemoaffinity-determined fiber connection scheme as outlined above.

Selective Growth in Peripheral Nerves

The demonstration that growing fibers may preferentially enter and follow particular trails to reach their destinations in the central nervous system raises again the question of whether anything of the kind may occur in the regeneration or initial growth within the fiber systems of the peripheral nerves. The evidence in the past has seemed to exclude such selectivity (Weiss and Edds, 85; Guth, 16; Weiss and Hoag, 86; Weiss and Taylor, 88) but the question has been studied thus far only in a very narrow range of conditions and deserves further investigation. There is a great wealth of possi-

bilities for testing; the procedures are simple, and the results come quickly. Incidental observations in a variety of nerve regeneration studies leave this writer with the impression that some selectivity may exist in the regrowth of fibers into degenerated nerve trunks at least on a gross level in the lower vertebrates—as between sensory and motor channels, or between pectoral and pelvic fin nerves. Selectivity on a more refined scale is not excluded.

The course that nerves follow in entering transplanted aneurogenic limbs, as in the experiments of Piatt (35), are strongly suggestive that the general fiber patterns formed in the peripheral nervous system are determined in part at least by chemical affinities. The embryonic formation of the trochlear, as well as of other cranial nerves (Hamburger, 19) is difficult to explain in many cases without assuming selective chemical guidance. It is logical that the addition of any new fibers to peripheral nerves at the later stages of development when the fiber systems are already well formed by preceding nerve outgrowth, would be much more effective if the late-comers were to follow their appropriate channels than if they were obliged to explore at random until the correct end organs were located.

Gradient Plasticity

An apparent deviation from the results of ablating half of the retina in goldfish has been reported recently by Gaze, Jacobson, and Szekely (15). Optic nerves which are composed of a double supply of temporal fibers (obtained by replacing, in embryonic stages, the nasal half retina by a transplanted temporal retina) apparently formed synaptic connections throughout both halves of the tectum instead of leaving one half uninnervated. Both half retinas were mapped as an orderly single half field but the half field was spread across the whole tectum as if the recipient tectal half field had expanded to include tectal cells labeled originally for nasal retina. It is not inconceivable that the dynamics and biochemistry of the morphogenetic field allow for this kind of plasticity in gradient organization. Before we invest in possible explanations of how a half tectum tagged for nasal fibers can be relabeled for temporal fibers under these conditions, there is an alternative possibility to be ruled out. Hyperplasia and hypertrophy beyond the normal limits are to be expected in the half tectum that receives a double ingrowth of temporal fibers. Conversely, atrophy and hypoplasia are to be expected in the uninnervated half (Kollros, 28). Some shrinkage and swelling accompany denervation and reinnervation of the adult tectum, but the effects are much greater during development. The combination of the resultant atrophy in one half and hypertrophy in the other half might well be responsible for the observed effect obviating the chemical relabeling problem.

CHEMOTROPISM IN REINNERVATION OF OPTIC TECTUM. We are currently trying to test the strength and nature of the selective growth forces in the optic system of fishes, mainly by surgical and histological methods that involve various types of displacement of the central fiber bundles as they approach and enter the optic tectum. When the medial and lateral bundles are cut and crossed at the anterior pole of the tectum shortly beyond the point where they diverge, the crossed bundles regenerate and promptly recross against

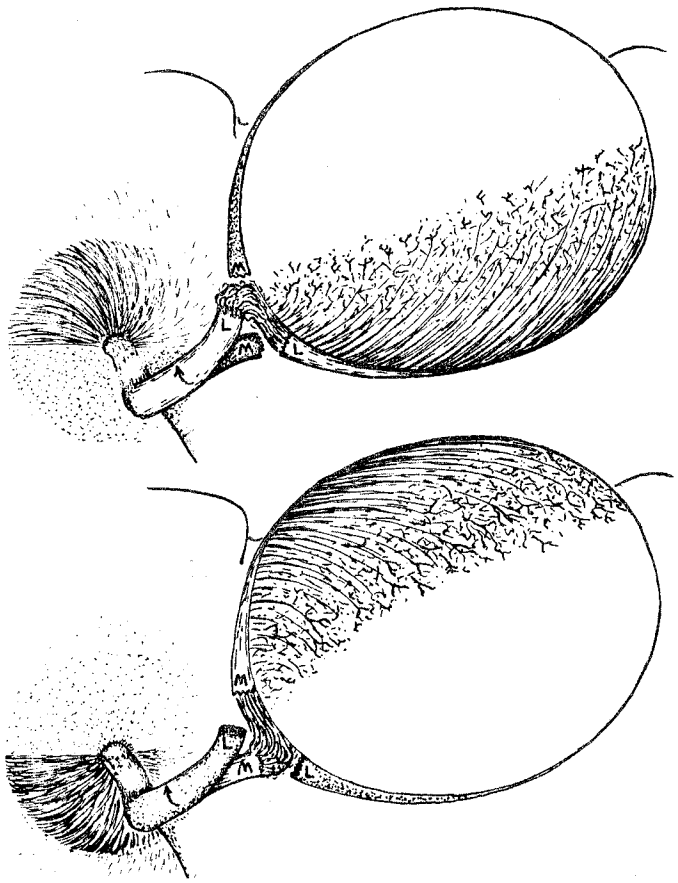


Fig. 6.6 Experiment in which the medial and lateral bundles of the optic nerves are cut and crossed. Regenerating fibers do not enter the tectum indiscriminately or along lines of least resistance, but re-cross to enter their proper channels. (After Arora and Sperry, *Am. Zool.* 2:389, 1962.)

the imposed mechanical biases to reenter their own proper channels, as shown in Figure 6.6 (Arora and Sperry, 4, 5). In other experiments the lateral bundle is cut long and crossed far up into the emptied channel of the medial bundle. Under these conditions the displaced lateral fibers again avoid connections in the dorsal tectum and manage to get back to the ventral tectum (Arora, 1; Arora and Sperry, 4). They do this, however, not by backtracking, but by taking a short cut across the dorsal tectum through the parallel layer. In doing so the optic fibers build a heavy fiber system across the equator of the tectum that normally is not present. In highly delicate surgery, Arora has been able to deflect the teased-out posterior fascicle of the lateral bundle across the midline into the medial bundle of the contralateral tectum. Again, the fibers find their way back to the appropriate posteroventral sector of the tectum, but on the contralateral side this time and superimposed on the normal innervation of that side. In part, the lateral fibers mingle with the medial fibers running parallel with them in the parallel layer of the tectum, but they then push onward across the equator to attain their proper posteroventral synaptic zone. Numerous variations of these surgical derangements produce similar and intermediate growth patterns illustrating

the same directive tendencies. It is a common and repeated observation that fibers growing through the parallel layer by-pass those regions of the tectum inappropriate for an orderly mapping of the retinotectal projections under conditions where the mechanical opportunities are equal for different fiber types.

Further behavioral indications of the refinement and selectivity of the regeneration process have been obtained in experiments just completed in our laboratory by Limpo. Fish were trained by the jumping technique to discriminate between fine shades of color in the yellow-green, green-blue, and yellow-red ranges in different cases. The thresholds for such discriminations were determined for normal fish and for fish with regenerated optic nerves and found to be of the same order. Also, thresholds tested before optic nerve section and regeneration were not significantly different from those tested on completion of regeneration in the same individuals.

There remain a host of unanswered problems in this field, which we have only begun to investigate. Attempts to study the regrowth of optic axons from smaller and smaller retinal remnants have so far been discouraging for anything smaller than about a third or a fourth of the retinal field. We have been unable, thus far, to obtain satisfactory staining of smaller regenerated fascicles after their entrance into the tectum. The reasons are not clear.

Application to Mammalian Visual System

I have indicated elsewhere how the gradient, chemotactic, and related concepts, based on data from the lower vertebrates, might be useful in explaining the ontogenetic organization of the primate visual system (Sperry, 63). The extensive and refined specification of the optic fibers is most clearly laid out in the lateral geniculate nucleus of man where the visual half field is mapped six times, in layer-cake fashion. Each map is in register with the others, with three layers for each eye and each pair of layers contains different cell types. Just as the optic fibers find their correct terminal zone in the tectum or colliculus, they must similarly find their proper locus on the geniculate surface. Each fiber then dips downward to connect in only one layer, as a rule, and only in the layer appropriate for the eye of origin, by-passing the neurons of other layers.

Presumably all of the refined selectivity that is histologically evident in the lateral geniculate nucleus is retained and more is added in the striate cortex, although in the cortex the different cell types are not so distinctly and visibly segregated. Projection from geniculate to striate cortex seems to involve a slight change in gradient relations in that the cortical units are found to pick up from an elongate field instead of the more circular receptive fields found at the geniculate level (Wiesel and Hubel, 89). The embryogenesis of such a system may be explained tentatively on the assumption that a given cortical unit accepts radiation fiber terminations rather loosely with respect to one or more chemical gradient factors in the fiber system while exercising more strict selectivity for others. The distribution of the loose or sliding affinities would thus reflect the distribution of the cortical unit tilts, and vice versa. The nature of this distribution pattern remains to be worked out, as do the developmental

forces that govern it. One would suspect a lateral inhibitory surround effect operating in the differentiation of the various cortical columns to insure a proper distribution of different direction indicators.

Endogenous Physiological Properties

The foregoing discussion of behavioral nerve nets has been concentrated almost entirely on the matter of the patterning of the fiber connections. However, the functional proficiency of the neural circuitry in operation depends not only on the interneuronal contacts and the network diagram, but also on the diverse physiological properties of the various cellular units of which the circuits are composed. The neurons and their fibers act as conductors, but they also appear to serve in a variety of other specialized roles as pacemakers, amplifiers, triggers, tuners, timers, sensors, rectifiers, relays, secretors, and other elements that depend on a correspondingly wide diversification in the endogenous physiological and cytochemical properties. The resting excitatory threshold may be intrinsically high or low, stable or fluctuating in various ways in different cell types (Bullock, 10). The neuron may discharge in bursts or in trains, either of which may vary characteristically to give a wide variety of specific pulse pattern properties (Strumwasser, 68). Neurons may differ also in properties determining their pickup or pulse-pattern detector capacities. In this connection it should be understood that there is nothing in the above discussion intended to be critical of impulse specificity as a factor in central integration except in the particular context in which it was earlier applied in the resonance explanation of homologous response. It remains entirely possible that pulse specificity plays a significant role in other integrative functions, though at present rather little is known about this in the vertebrate nervous system.

All of the specialized endogenous physiological properties of the various neuron types presumably arise, like the specificities that govern the morphological characteristics and the growth of connections, from cytochemical differentiation of the neuron population during development. They too, like the fiber connection patterns and more gross anatomical features, are thus dependent on genetic control and are, to a large extent, a matter of inheritance.

Functional Shaping of Nerve Circuits

It is obvious that there are endless problems remaining for future analysis in the developmental organization of the nerve circuits of behavior. Some of the problems have special importance because of implications that go far beyond the concerns of organogenesis per se. These perhaps merit some further comment here, even though the experimental evidence is sparse and most aspects are still rather speculative. One area in which more information will have widespread impact concerns the extent to which inheritance influences behavior, particularly in man. The counterpart of this question is the extent to which, and means by which, function shapes and reshapes the behavioral networks. In addition to the practical aspects are implications for theories of memory and learning and of their biological bases. These in turn are hardly separable from prob-

lems of perception, motivation, and other higher activities of the nervous system. The recent experiments of Hamburger (21) on the development of behavior in the chick, in which he is collaborating with a psychologist combining psychological and developmental approaches, point to a very promising trend for the future.

It is possible that the unknown changes imposed on the nervous system by learning and experience are distinct and removed from those processes one deals with in development and maturation. It is also possible, and not at all improbable, that the changes imposed by function are similar to, or a direct derivative of developmental features, and perhaps best understood in these terms. There are reasons to think that long-term memory in man could involve a specificity effect in the machinery for late neuron differentiation that gains behavioral expression through either a modification of the cell's fiber contacts or of its intrinsic physiological properties.

An interesting lead is found in the increasing evidence that some nerve cells are subject to disuse atrophy. If not adequately stimulated, these neurons may regress until permanently destroyed. Prisoners kept in "black cells" for several months come out totally blind (Walls, 75). Chimpanzees and other mammals raised in the dark show irreversible degenerative changes in the retina and visual pathways. If the same animals are raised in diffuse light, the atrophic effects are less severe, but apparently those neuronal units activated by edges and contrasts, which are not stimulated in diffuse light, undergo atrophy (Riesen, 39; Wiesel and Hubel, 89). If disuse effects of the same kind obtain farther centrally among associational neurons of the cortex (see Held and Hein, 24), the result would be more nearly like that of learning. Many elements deeper in the brain centers must discharge only in very special activities, and, if these activities are not exercised—especially during maturational stages when the neurons seem to be particularly dependent on use—the neuron types involved may regress, leaving profound functional deficiencies in the integrative machinery.

One can arbitrarily distinguish use effects that consist of stamping in and preserving neural organization developed in growth, from use effects that add new organization, anatomical or physiological, to the developed system. Despite common impressions to the contrary (especially in psychology) it appears to be mainly the former that has been involved in most of the sensory deprivation studies to date (Sperry, 55). The above distinction tends to break down, however, in the central association areas, where the growth pressures are more diffuse. It is not clear at present to what extent fractional parts of a neuron, like synaptic endings, and separate dendritic branches, spines, or other elements may undergo disuse atrophy, leaving other parts of the same neuron normally functional. The so-called "disuse" effects, described by Cajal (37) from histological studies, appear to be more a reflection of interneuronal trophic dependencies than a dependency on excitation.

Problems relating to the use-dependent properties of nervous elements add up to a whole field in itself, with important ramifications in psychiatry, ethology, and other disciplines concerned with the effects of inheritance and

early experience on adult function. It would be no surprise to find that the neural basis of imprinting is a direct evolutionary elaboration of the physiology or biochemistry of the above-mentioned use effects in the neural networks. Furthermore, it remains an open question whether the effects of function—that is, learning and memory—add or subtract any actual fiber structures or synaptic connections to the established morphology. It is possible, though not particularly indicated, that the neural changes implanted by learning and memory are essentially physiological in nature; membrane or other micro or molecular changes could affect excitatory threshold, conductance, and resistance to impulse transmission, or endogenous discharge properties all within the already established morphological networks. Between the strictly inherited organization of the behavioral networks and the strictly acquired, we recognize an important intermediate realm of nervous development in which function and growth go on simultaneously with mutual interactions. The anatomical effects of functional influence during these stages may not be large or even visible under the light microscope, but the minute differences may be critical in terms of behavior, especially with reference to human childhood.

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